The synthesis of IFN-β during genital tract *Chlamydia muridarum* infection is important in minimizing the genital tract pathology caused by the immune responses to chlamydial infection

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INTRODUCTION

Chlamydia trachomatis (*Ct*) is the most common bacterial sexually transmitted pathogen and is a significant threat to the reproductive health of women. With an estimated 2.8 million new infections domestically and 131 million new *Ct* infections acquired annually globally, chlamydia is costing health care systems billions of dollars to treat not only the acute infections but also the complications they cause. *Ct* genital tract infections are associated with cervicitis, urethritis, and endometritis; complications from chronic infections include pelvic inflammatory disease (PID) and its sequelae of chronic pelvic pain, ectopic pregnancy, and tubal infertility. Although treatable with antibiotics, individuals infected with *Ct* are often unaware of the infection, and the asymptomatic nature of the disease facilitates the spread of the bacterium through further sexual contact with uninfected individuals. As a result, *Ct* infections have continued to increase over the past two decades, despite the implementation of screening and early intervention strategies.

OBJECTIVES

Our long-term goal is to understand the pathophysiologic processes that contribute to *Chlamydia*-induced reproductive tract pathology. We are focused on identifying the inflammatory mediators that induce scarring of the oviduct epithelium and identifying therapeutic counter-measures that can prevent this. We were the first to demonstrate, and others have now confirmed, that TLR3 is a key pattern recognition receptor (PRR) in the immune response to *Chlamydia muridarum* (*Cm)* in mice.

Controversy regarding the role of IFN-β in *Chlamydia* pathogenesis: The exact role of IFN-β and its contribution to the overall immune response to *Chlamydia* infection is unclear. Experiments conducted in mice defective in the interferon alpha-beta receptor (IFNAR) suggest that Type-1 IFNs are detrimental to the host in both the genital tract and lung infection models. In contrast, our data suggest a different role for IFN-β in the context of TLR3-deficiency: Our preliminary data show that TLR3-deficiency results in defective IFN-β synthesis, increased *Chlamydia* replication, and that oviduct epithelial (OE) cells derived from TLR3$^{-/-}$ mice exhibit significant reductions in the expression of several inflammatory cytokines and chemokines. Because the diminished *C. muridarum*-induced IFN-β synthesis is one of the possible contributors that will make TLR3$^{-/-}$ mice more susceptible to *Chlamydia*-induced pathology, our contrasting findings suggest that ***IFN-β is important for an effective immune response to Chlamydia infections and is thus beneficial to the host.***

METHODS

To conclusively determine whether IFN-β is either beneficial or detrimental to the host during genital tract *Chlamydia* infection, we examined the course of *C. muridarum* (*Cm*) infection in wild-type versus mice deficient in IFN-β synthesis via the following methodology:

1. Vaginal sponges to determine the effect of IFN-β's absence on cytokine secretion into the genital tracts of *C. muridarum* infected mice
2. Flow cytometry for analyzing the impact of IFN-β on total T-cell populations in the genital tract of *C. muridarum* infected mice
3. Vaginal swabs to determine the effect of IFN-β's absence on *Chlamydia* clearance
4. Gross and microscopic examination of genital tract tissue to determine oviduct pathology (with emphasis on hydrosalpinx, inflammatory induced damages, and oviduct dilatation).

RESULTS

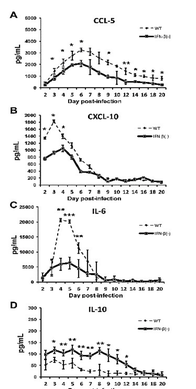

Fig 1. Genital tract secretions were obtained from *Cm*-infected WT and IFN-β(-) mice during the first 20 days post-infection, and analyzed by multiplex cytokine assay for: **(A)** CCL-5, **(B)** CXCL-10, **(C)** IL-6, and **(D)** IL-10 synthesis. Data are from a representative experiment where *n=8* mice. *= *p <0.05*; **= *p <0.005*; ***= *p <0.001*

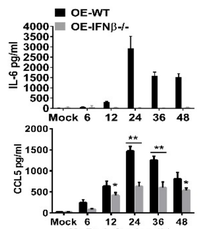

Fig 2. ELISA was used to measure *Cm*-infection-induced **(A)** IL-6 and **(B)** CCL-5 secreted into the supernatants of OE cells isolated from wild-type and IFN-β KO mice at 24h PI. Data presented is representative data from four different experiments. Significance was determined using standard two-way Anova; *= *p value <0.05*; **= *p value <0.005*.

IFN-β deficiency dysregulates the *Chlamydia*-induced syntheses of multiple inflammatory mediators:

• We infected groups of 10 wild-type (C57BL/6NJ mice) and 10 IFN-β KO mice intra-vaginally with 10^5 IFU *Cm* 7 days after treatment with *Depo Provera*, and we measured the syntheses of several cytokines in the first 20 days post-infection by multiplex ELISA (**Fig 1**).

• To confirm the multiplex data and to ascertain if the epithelial cells lining the lumen of the oviduct responded to *in vitro Cm* infection in a manner reflective of the *in vivo* findings, we isolated OE cells from WT and IFN-β KO mice and infected the cells with 5 IFU/ cell *Cm*. As shown in **Fig 2**, the *Cm*-induced syntheses of IL-6 and CCL5 were both significantly lower in the supernatants of the IFN-β(-) OE cells at 24hrs post-infection in our standard ELISA assays.

Cm-induced IFN-β synthesis has an impact on genital-tract T-cell populations:

• To examine the impact of IFN-β on T-cell recruitment into the genital tract during *Cm* infection, five WT and five IFN-β KO mice were infected intravaginally with 10^5 IFU *Cm* before being sacrificed at either day 7 or day 21 of infection and their lower genital tracts processed for flow cytometry. As shown in **Fig 3,** the percentage of CD4+ T-cells were lower in the genital tracts of *Cm*-infected IFN-β KO mice at both day 7 and day 21; however, it was only statistically significant at day 7. The CD8+ T-cell percentages trended lower at both day 7 and day 21 in the IFN-β KO mice as well; however, the amount lower at day 21 only was statistically significant.

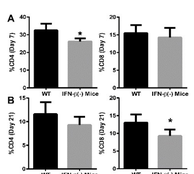

Fig 3. The percentages of CD4 and CD8 T-cells from each group on **(A)** Day 7 and **(B)** Day 21 were summarized in the graphs. Data shown are representative results from two independent experiments with 3 to 4 mice per time point. Bar graphs show mean number ± SD. Differences between groups for each parameter were determined by *t*-Test *= *p <0.05*.

IFN-β is required for more efficient clearance of *Cm* from the genital tracts of infected mice:

• We infected groups of 10 WT and 10 IFN-β KO mice intra-vaginally infected with 10^5 IFU *Cm* 7 days after treatment with *Depo Provera*, and we examined the impact of IFN-β deficiency signaling on chlamydial shedding. As shown in **Fig 4**, *Cm* was virtually eliminated by day 42 in the WT mice but was still detectable in the genital tracts of IFN-β KO mice. Additionally, the IFN-β KO mice shed significantly more *Cm* throughout infection than the wild-type mice.

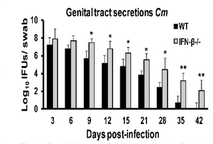

Fig 4. Genital tract infections were performed, swabs were collected until day 42. *C. muridarum* titers were determined by infecting fresh McCoy cell monolayers. Combined data, *n=18* mice for WT; *n=17* mice for IFN-β$^{-/-}$. IFU= inclusion forming units. *= *p <0.05*; **= *p <0.005*.

IFN-β deficiency leads to more severe late-stage genital tract pathology:

• Groups of 10 WT and IFN-β KO mice were intravaginally inoculated with 10^5 IFU *Cm* for qualitative histological evaluation of lesions in the lower and upper genital tract at day 56 of infection by microscopy and quantitatively scored by a pathologist on a 0-4 scale. **Fig 5** shows a chart summarizing the histological changes that are related to IFN-β deficiency. Collectively, these findings showed that IFN-β deficiency can lead to more pronounced chronic sequelae, such as uterine horn dilatation and oviduct hydrosalpinx, during late stages of *Cm* genital tract infections in mice.

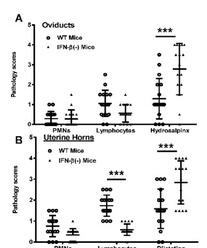

Fig 5. Histopathological changes of both uterine horns and oviducts from WT and IFNβ(-) mice. These results are from combined experiments where *n=17* mice for WT and *n=16* mice for IFN-β(-) mice. Differences between groups for each parameter were determined by two-way *Anova*. ***= *p <0.005*.

Summary and Ongoing Studies:

• Our data show that IFN-β has a host beneficial role in the immune response to genital tract Chlamydia infections, challenging the paradigm that type-1 IFN is detrimental established by other investigators.
• We are currently repeating *in vitro* experiments in OE cells to complete manuscripts and grant submissions.
• We are awaiting completion of a novel CRISPR KO mouse line that is defective in IFNα expression to assess its role in genital tract Chlamydia infections

ACKNOWLEDGEMENTS:

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